ESTIMATION OF LARVAL INSTARS OF *HYPSIPYLA ROBUSTA* MOORE (LEPIDOPTERA: PYRALIDAE) BY LARVAL FRASS WIDTHS

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Abstract

Frass widths are used to estimate the larval instars of *Hypsipyla robusta* Moore, a shoot borer of Australian red cedar *Toona australis* (F. Muell.) Harms. Based on the relationship betwen larval frass widths and larval head capsule widthe, the inter-instar boundaries of larval frass widths are calculated. The average frass widths of individual larvae are then compared with the boundaries to determine the larval instars. Ninety-three percent of laboratory larvae were assigned to their correct instars by frass widths and 87% of field larvae were estimated as in the same instars by frass widths as that determined by head capsule widths.

Introduction

The Cedar tip moth *Hypsipyla robusta* Moore is a serious pest of a number of Meliaceae species, including Australian Red Cedar *Toona australis* (F. Muell.) Harms.* (Beeson 1919). The larvae feed inside various tissues of the host plant, especially the growing shoots and pupate inside larval tunnels (Beeson 1919). Due to their cryptic nature, the development stages of larvae cannot be determined directly. As an indirect approach, this paper explores the possibility of using larval frass widths (FW) to estimate larval head capsule widths (HCW) and therefore larval instars. Such a technique may be used by forresters in fine-tuning the timing of control measures against this pest.

Frass of *H. robusta* larvae is found as conspicuous clumps at the openings to the larval tunnels (Roberts 1968). The inside of the tunnels, however, contains little frass (personal observation), suggesting that fresh frass is constantly being pushed out. It is therefore possible to relate the exterior frass to the current development stages of the larvae.

Methods

Larvae were obtained from a laboratory stock originating from mature larvae collected in a red cedar plantation in Macksville, NSW and maintained on the artificial diet of Couilloud and Guiol (1980). To enhance feeding, a small amount of macerated fresh young red cedar shoots was incorporated into the diet. Larvae from the original site were incorporated into the stock at least twice a year.

One hundred newly-hatched larvae were reared separately in glass vials (50x12 mm) until pupation. The instar of a larva was determined from the number of head capsules it shed. 1st to 3rd instar larvae were fed with the terminal parts of young shoots whilst the older larvae were supplied with cuttings

*The name *Toona ciliata* M. Roem may soon replace *Toona australis* (F. Muell.) Harms.

from the stouter parts of young shoots, in accordance with their natural feeding habits (personal observation). Food was replaced every 1-3 days, depending on the consumption rate and freshness of the tissue. Frass was removed daily from the glass vials. Rearing was in a room with temperature at 26±1°C and light period at 14L:10D. Humidity was not controlled, the room maintained humid by a vaporiser (KAZ Model 76).

At least 20 larvae at every instar were measured for HCW and FW, to the nearest 1/40 mm, under a stereo microscope fitted with an eyepiece scale. Due to the frequent rupture of head capsules in the last moult, the HCW's of the last instar larvae were replaced with the corresponding larval head widths just before pupation. For each larva measured for HCW, 20 air-dried frass pellets produced by that larva were measured and the mean FW calculated.

A separate set of data involving 30 larvae was collected in a red cedar plantation at Macksville, NSW, to test the effectiveness of FW in estimating larval instars in the field. The frass was transfered from the infested shoots to glass vials and then the shoots were dissected for larvae. The frass and the associated larvae were taken back to the laboratory and the larvae were further reared to obtain the head capsules for HCW measurements.

Results

Larvae moulted either 5 (32%) or 6 (68%) times before pupation, as noted previously by Atuahene and Souto (1983). A recent study by the authors showed 5- and 6-instar forms in larvae of both sexes (82% and 75% of 6-instar forms in males and females respectively), hence the variation in the number of larval instars is not likely to be sex dependant.

Larvae of the 5- and 6-instar forms showed similar HCW ranges in the 1st to 5th instars. Hence data were pooled and the joint mean and ranges are given in Table 1. Total separation was achieved by HCW for the first 4 instars, whereas the 5th and 6th instar larvae showed some overlapping in their HCW ranges. Further examination of the data showed that amongst the 25 5th instar larvae measured, only one had its HCW range fall within that of the 6th instar larvae. Thus HCW can still be considered a reliable predictor of larval ages. The inter-instar boundaries in HCW for any two non-overlapping instars were arbitrarily determined as the average of the maximum HCW of the former instar and the minimal HCW of the following instar, with that for the 5th and 6th instars as the minimum HCW of the 6th instar (Fig. 1).

Overlapping in FW started in the 4th instar and the relative within-instar variations (expressed as SE/mean) were consistently higher than that in HCW (Table 1). However, FW showed apparent positive correlation with HCW (Fig. 1) and the correlation was significant ($t=51.37$, $df=133$, $p<0.001$). The relationship was well fitted by linear regression (Fig. 1). Assuming the regression equation correctly described the true realtionship between HCW and FW, the inter-instar boundaries of FW obtained by supplanting the HCW

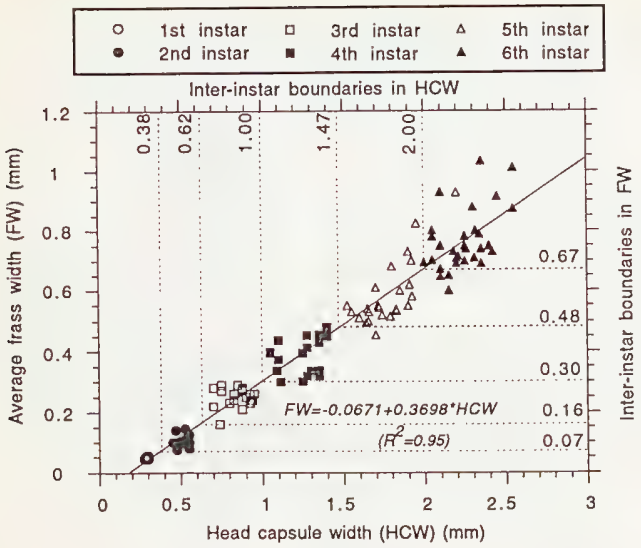

Fig. 1. The relationship between average frass width (FW) and larval head capsule width (HCW) in *Hypsipyla robusta*, with their inter-instar boundaries shown as dotted lines. Data from larvae reared on host plant material. (See text for details).

boundaries into the equation (Fig. 1) should perform equally well in delimiting larval instars. In effect, the percentages of correct estimations of larval instars by comparing individual FW's with the FW boundaries were 100% for the first 3 instars, 95% for the 4th instar, 76% for the 5th instar and 90% for the 6th instar. Overall, 93% of the measured larvae were assigned to their correct instars by their FW's. Most of the misclassifications occurred in the 5th and 6th instar, which is probably due to the overlapping of the HCW ranges of these two instars.

With field data, 26 larvae (87%) were assigned to the same instars by both HCW and FW. Two larvae that were assigned to the 5th instar by HCW were estimated as 6th instar by FW and 2 larvae that were determined as 5th instar by HCW were estimated as 4th instar by FW.

Discussion

The above analysis demonstrates that FW is a useful predictor of larval instars of *H. robusta*, especially for the first 4 instars. The degree of predictiveness is comparable with that of HCW. Since FW data are more easily accessed than HCW data, the FW approach appears promising. When applied to field situations, care should be taken to measure only those

Table 1. Larval head capsule widths (HCW) and frass widths (FW) in *Hypsipyla robusta*.

larval instar	head capsule width (HCW)		frass width (FW)	
	mean±SE(n) (SE/mean)	range	mean±SE(n) (SE/mean)	range
1st	0.29±0.01 (20) (0.03)	0.28-0.30 (0.05)	0.05±0.00 (20)	0.05-0.06
2nd	0.49±0.04 (20) (0.08)	0.45-0.53	0.10±0.02 (20) (0.20)	0.08-0.15
3rd	0.85±0.08 (20) (0.09)	0.70-0.95	0.25±0.03 (20) (0.12)	0.16-0.29
4th	1.24±0.13 (20) (0.10)	1.05-1.40	0.39±0.06 (20) (0.15)	0.30-0.48
5th	1.78±0.15 (25) (0.08)	1.53-2.20	0.59±0.11 (25) (0.19)	0.46-0.93
6th	2.25±0.15 (30) (0.07)	2.00-2.25 (0.13)	0.77±0.10 (30)	0.60-1.02

frass pellets of apparently larger sizes to minimise the possibility of accidentally including frass pellets produced at earlier developmental stages. The number of frass pellets required varies with instars. Under the assumption of normal distribution of FW, a minimal number of 16 frass pellets is recommended to keep the relative sampling error below 10%. Finally, the inter-instar boundaries of FW given here are based on larvae reared in an artificial environment. Although they were validated by one set of field data, further validation and possibly modification may be needed before widespread application of the method.

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